

Rec'd PCT/PTO 22 APR 2005



10/532265 #1
PCT/GB 2003 700 4651



INVESTOR IN PEOPLE

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

REC'D 16 DEC 2003

WIPO PCT

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

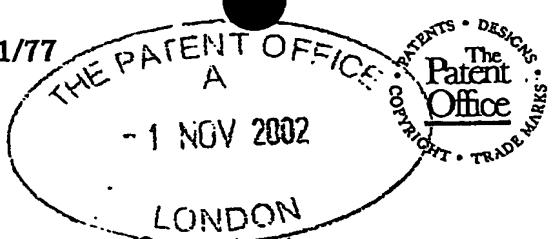
Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Dated

10 November 2003

BEST AVAILABLE COPY

04NOV02 5760518-1 D02246
P01/7700 0.00-0225553.7

177

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

 Cardiff Road
 Newport
 South Wales
 NP10 8QQ

1. Your reference

P015153GB

2. Patent application number

(The Patent Office will fill in this part)

0225553.7

01 NOV 2002

3. Full name, address and postcode of the or of each applicant (underline all surnames)

 Medical Research Council
 20 Park Crescent
 London
 W1B 1AL

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

S9600700

4. Title of the invention

Prion Decontamination

5. Name of your agent (if you have one)

D Young & Co

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

 21 New Fetter Lane
 London
 EC4A 1DA

Patents ADP number (if you know it)

59006

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country

Priority application number
(if you know it)Date of filing
(day / month / year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing
(day / month / year)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if

Yes

- a) any applicant named in part 3 is not an inventor, or
- b) there is an inventor who is not named as an applicant, or
- c) any named applicant is a corporate body.

See note (d))

ents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form.
Do not count copies of the same document

Continuation sheets of this form 0

Description 19

Claim(s) 2

CF

Abstract 1

Drawing(s) 4 *4*

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (*Patents Form 7/77*)

Request for preliminary examination and search (*Patents Form 9/77*)

Request for substantive examination
(*Patents Form 10/77*)

Any other documents
(please specify)

11.

I/We request the grant of a patent on the basis of this application.

Signature *D Young & Co*
D Young & Co (Agents for the Applicants)

Date 31 October 2002

12. Name and daytime telephone number of person to contact in the United Kingdom

Charles Harding

023 8071 9500

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

- a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 08459 500505.
- b) Write your answers in capital letters using black ink or you may type them.
- c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.
- d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.
- e) Once you have filled in the form you must remember to sign and date it.
- f) For details of the fee and ways to pay please contact the Patent Office.

PRION DECONTAMINATION

Field of the Invention

The present invention relates to methods and reagents for use in prion decontamination. In particular, the invention relates to prion decontamination of surgical instruments.

Background to the Invention

The persistence and resistance of the prion agents responsible for vCJD has raised fears about the possibility of iatrogenic transmission following surgery. The prion diseases, which include scrapie in sheep, BSE in cattle and CJD in humans are a novel group of transmissible, fatal neurodegenerative conditions. The transmissible agent termed a prion is comprised largely or solely of a conformational isomer of a normal cellular PrP protein. This conformer designated PrP^{Sc}, has several unusual properties including resistance to proteolysis, detergent insolubility and high thermal stability. These physical properties coupled to recent observations that PrP^{Sc} adheres strongly to surgical steel present problems in the cleaning and sterilisation of surgical instruments as prion infectivity is known to be resistant to conventional autoclaving.

In the absence of a pre-clinical diagnostic test for vCJD pre-surgical testing of patients is not possible. Although in a minority of cases where CJD is suspected or confirmed used instruments can be quarantined or destroyed, for the majority of procedures new methods of decontamination are required. The UK Department of Health is currently engaged in several research projects in order to try to address the problem of iatrogenic CJD transmissions.

Conventional studies have shown survival of prions under autoclave conditions.

30

Commercial reagents currently in use for cleaning of surgical instruments prior to

autoclaving have little or no effect upon PrP^{Sc} contamination.

Existing methods of decontamination such as those involving LpH, LpHse, and Endozyme Plus are of limited use in destroying infectivity.

5

Taylor 1999 (Taylor D.M., *Inactivation of prions by physical and chemical means*. 1999, J. Hosp. Infect. 43, 69-76.) discloses the use of sodium hypochlorite solutions and 2M sodium hydroxide in prion inactivation. However, there are problems with this approach such as incomplete inactivation. Furthermore, resistance of prions to 10 autoclaving is reported.

The present invention seeks to overcome problem(s) associated with the prior art.

Summary of the Invention

15

The inventors have developed a combined detergent and proteolytic enzyme treatment that reduces the titre of prion infectivity. In some embodiments reduction by about 1 million fold is achieved. The reagents used are water soluble, stable and of low toxicity. The protocol for their use is compatible with existing hardware such as 20 machines used for pre-washing medical instruments prior to autoclaving. Thus the invention provides methods and reagents by which entities such as medical instruments can be decontaminated of prion infectivity.

The methods of the present invention, in particular the combination methods, are 25 effective for prion inactivation such as vCJD prion inactivation. The methods find particular application on surfaces such as metal surfaces.

The methods and compositions of the present invention such as reagents for addition to hospital instrument washing machines find application in hospital or sterile services 30 providers throughout the world to reduce the possibility of prion contamination such as that leading to iatrogenic CJD.

Advantageously, the methods of the present invention are used in addition to, and preferably preceding, autoclaving.

It is surprisingly shown herein that vCJD prions are relatively thermolabile compared to other prions. This surprising finding allows enhancement of the methods of the present invention as described below.

Without wishing to be bound by theory, the present invention makes use of specific knowledge of PrP^{Sc} chemistry. The inventors formulated the rationale that treating (eg. boiling) with detergent such as SDS should render PrP^{Sc} more susceptible to proteases. Numerous proteases and conditions were then examined which further led to the invention of the methods of the present invention.

Detailed Description of the Invention

15

In one aspect, the present invention provides a method for prion decontamination by enhancing the destructive effect of autoclaving. In this aspect, the present invention provides a method comprising (i) contacting an entity to be decontaminated with a detergent such as SDS, and (ii) autoclaving said entity.

20

In another aspect, the invention provides a method for prion decontamination comprising (i) contacting an entity to be decontaminated with a detergent, (ii) contacting said entity with a protease, and optionally (iii) autoclaving said entity.

25

In another aspect, the invention provides a method for prion decontamination comprising (i) contacting an entity to be decontaminated with a detergent, (ii) contacting said entity with a first protease, (iii) contacting said entity with a second protease, and optionally (iv) autoclaving said entity.

30

Preferably said first and second proteases are different. The entity may be contacted with the first and second proteases simultaneously or sequentially. Preferably the entity is contacted with the first and second proteases sequentially.

5 Optionally, further protease treatment steps may be incorporated in the methods of the present invention. Thus, in another aspect, the invention provides a method for prion decontamination comprising (i) contacting an entity to be decontaminated with a detergent, (ii) contacting said entity with a first protease, (iii) contacting said entity with a second protease, (iv) contacting said entity with one or more further proteases, and optionally (v) autoclaving said entity.

Proteases

10

Temperature/protease concentration optima:

15 As is plain to a person of ordinary skill in the art, the higher the concentration of protease(s), the greater and more rapid destruction is achieved. Combinations of protease concentration and time may be chosen according to need. These can be optimised by routine trial and error.

20

Examples presented herein include conditions optimal for use in automated washing machines. Furthermore, the conditions chosen are advantageously low in cost.

25 Incubation temperatures for the protease varies according to the protease used. Generally, any temperature from room temperature (eg. 20°C) up to 60°C is acceptable. The preferred temperature for papain is 30°C, the preferred temperature for ProteinaseK is 55°C, the preferred temperature for pronase is 45°C, the preferred temperature for Bromelain is 40°C. As the temperature moves away from the optimum for a particular protease, deactivation takes longer. Clearly, this can be compensated for by incubating for a longer time or using a greater concentration of protease. At temperatures above 60°C activities can be lower and the enzymes can become inactivated, but clearly individual protease preparations will have individual 30 deactivation temperatures and the manufacturers' guidance should be followed wherever possible.

Proteases can be adversely affected (eg. suffer reduced activity or loss of activity) in the presence of excess detergent. Individual proteases have individual characteristics, and it is well within the abilities of a person skilled in the art to avoid loss of activity due to detergent action. Manufacturers' guidance should be followed wherever possible. Advantageously detergent level(s) are reduced to so as not to significantly inhibit protease activity before/at the time of contact with protease.

The protease can be mixture of proteases. However, it should be noted that when contacting with several proteases at once, individual activities can be reduced and compensation might be necessary eg. by longer time of contact. This is discussed in more detail below.

Preferably the protease comprises one or more of ProteinaseK, Pronase, Papain, or Bromelain.

When the protease is bromelain, preferably detergent is substantially absent at the protease step.

Where at least two protease steps are used, preferably at least one of the proteases comprises ProteinaseK.

When only a single protease step is used, preferably the protease comprises ProteinaseK, pronase or papain, more preferably ProteinaseK or pronase, more preferably ProteinaseK.

Preferably at least two protease steps are used.

Preferably at least one detergent step and at least two protease steps are used.

In a one aspect the invention provides a method for prion decontamination comprising (i) contacting an entity to be decontaminated with a detergent, (ii) contacting said entity with pronase, and optionally (iii) autoclaving said entity.

In a preferred aspect the invention provides a method for prion decontamination comprising (i) contacting an entity to be decontaminated with a detergent, (ii) contacting said entity with ProteinaseK, and optionally (iii) autoclaving said entity.

5

In a preferred aspect the invention provides a method for prion decontamination comprising (i) contacting an entity to be decontaminated with a detergent, (ii) contacting said entity with pronase, (iii) contacting said entity with papain, and optionally (iv) autoclaving said entity.

10

In a preferred aspect the invention provides a method for prion decontamination comprising (i) contacting an entity to be decontaminated with a detergent, (ii) contacting said entity with ProteinaseK, (iii) contacting said entity with pronase, and optionally (iv) autoclaving said entity.

15

In a preferred aspect the invention provides a method for prion decontamination comprising (i) contacting an entity to be decontaminated with a detergent, (ii) contacting said entity with pronase, (iii) contacting said entity with ProteinaseK, and optionally (iv) autoclaving said entity.

20

Sequential/Simultaneous contact

Where more than one protease is used, the proteases may be combined into a single step. However, protease activity can be lowered in such an embodiment due to each protease digesting the other. Thus, the individual steps in the methods of the present invention are advantageously carried out sequentially for optimum efficacy. Furthermore, advantageously at least a proportion of the first protease is removed before the entity is contacted with the second or further protease.

25

Detergent step(s) and protease step(s) should advantageously not be combined as the detergent may inactivate the protease(s). Thus, advantageously at least a proportion of the detergent is removed (or diluted) before the entity is contacted with the protease in

order to maximise protease activity. Preferably the detergent step(s) precede protease step(s).

5 Detergent

Detergent is preferably an ionic detergent. Preferably the detergent is SDS.

Detergent may be used at any effective concentration. This may be easily determined
10 and/or optimised by trial and error. When the detergent is SDS, the final concentration
of the detergent with regard to the contacting with a detergent step is preferably about
2% to about 6%, preferably about 3% to about 5%, preferably about 4%, preferably
4%.

15 The entity may be contacted with the detergent at any suitable temperature. The
optimal temperature for the detergent step is flexible and is preferably at least 70°C,
preferably at least 80°C, preferably at least 90°C, preferably at least 100°C. The
temperature may be constrained by the nature of the entity, for example some medical
20 equipment such as endoscopes cannot tolerate high temperatures such as those used in
autoclaving. For these situations, the methods of the invention advantageously do not
involve autoclave conditions, and the temperature choice should be made by the
operator with regard to the tolerances of the entity being decontaminated. Examples of
methods according to the present invention which avoid the use of autoclave
25 conditions may be found in protocol B in the Examples section. Advantageously
methods according to the present invention such as those found in protocol B in the
Examples section may replace conventional prior art treatments such as LpH, LpHse,
and EndozymePlus treatment.

30 The time of incubation for the detergent step is flexible and is preferably at least 2
minutes. Protracted incubation at the detergent step can be advantageous, such as
hours or days or even longer.

Autoclaving

Autoclaving can be carried out following any suitable autoclave cycle. Typical cycles include 124°C for 15 minutes or 138°C for 15 minutes. Alternative cycles may be 5 chosen by the operator to suit their particular needs. Extended autoclave cycles may be advantageously employed.

Advantageously an autoclaving step is performed as a final step in the methods of the present invention. Combining the methods of the present invention with an 10 autoclaving step has the further advantage of minimising spread of infection *via* the entity being decontaminated such as surgical instruments. Furthermore, by combining with autoclaving in this manner, there may advantageously be a multiplicative increase in efficacy, ie. if each method can reduce infectious titre by 5 logs then combining them may reduce infectivity by even more, such as by 10 logs.

15

Entity to be decontaminated

The entity to be decontaminated may be any physical item for which it is desired to deactivate or remove prions. The term embraces solution(s) as well as objects such as 20 devices or medical instruments (including surgical instruments), particularly metal objects or part(s) thereof. The prions to be deactivated or removed may be in the entity (eg. in solution or suspension), or may be on the entity (eg. bound, attached or otherwise associated with a surface of the entity). Thus, the entity may comprise a surface. Said surface may be a surface of a medical instrument. Said surface may 25 comprise metal. Said metal may be steel such as surgical steel.

Decontamination

Decontamination refers to reduction in prion titre in a specific sample or setting. 30 Decontamination may refer to the removal of prions from a surface whether or not said prions are deactivated. Thus, decontamination includes deactivation and also includes the elimination of prions without regard to whether or not they are

destroyed/deactivated. When decontaminating, it is important that prion infectivity is removed from the surface or solution being decontaminated. This may be by destruction (deactivation) or by simple separation. The important aspect is that prions (ie. PrP^{Sc}) are no longer associated with the surface or solution being decontaminated

5 or are reduced in number and/or titre. Clearly, if non-infective prion fragments remain adhered to the surface after decontamination, this would not materially affect the decontamination or the fact that said surface had been successfully decontaminated.

10 Decontamination may be assessed by any suitable assay. Preferably, the assay used is western blotting or bioassay. Clearly assays such as bioassays and/or western blotting assays have a sensitivity limit. So long as prion titre (prion number/infectivity) has been reduced, then prion decontamination will be considered to have taken place.

15 Preferably prion decontamination is 100 fold, preferably 1000 fold, preferably 10,000 fold, preferably 100,000 fold, preferably 1,000,000 fold or even more. Preferably prions are completely eliminated or deactivated.

Assay methods

20 The reduction in prions produced by the methods of the present invention may be monitored by any suitable means known in the art. Specific examples of suitable assay techniques are provided herein to illustrate the assessment of prion reduction.

25 Clearly, certain methods will present themselves as more suitable for a given situation than other methods. For example, if prion decontamination is taking place in solution, then a western blotting approach might be most suitable. If prion decontamination is taking place on a surface, then direct visualisation on that surface might be most suitable. Alternatively for prion decontamination taking place on a surface, bioassay

30 might be the most suitable. Choice of individual assay methods for individual situations is well within the capabilities of a person skilled in the art. It will be appreciated that in many situations the most important indicator is loss / reduction of

infectivity. Currently, prion infectivity is most usually assessed by bioassay. However, biochemical assay of the infective conformer PrP^{Sc} is equally appropriate.

An example of a suitable monitoring method is an immunoblotting assay.

5 Advantageously the immunoblotting assay is, or is based on, the assay described in Wadsworth *et al* 2001 Lancet vol 358 pp 171-180.

An example of a suitable monitoring method is a bioassay. Bioassay methods are generally geared towards the individual prion species being assayed. Selection of

10 suitable bioassay methods is advantageously based on the prion species being assayed.

Kits

The present invention also relates to kits for use in decontamination. Thus the

15 invention provides a kit comprising detergent and a protease selected from the group consisting of ProteinaseK, papain, pronase, and bromelain. Preferably the kit comprises two or more such proteases. Preferably the detergent in the kit is SDS.

Compositions

20

The present invention also relates to compositions for use in decontamination. In one aspect, the invention provides a composition comprising an ionic detergent and one or more proteases selected from the group consisting of ProteinaseK, papain, pronase, and bromelain. In a preferred aspect, the composition comprises two or more such 25 proteases. In a more preferred aspect, the detergent of the composition is SDS.

The invention is now illustrated by way of examples which should not be regarded as limiting in scope. In the Examples, reference is made to the following figures:

30 Figure 1 shows a western blot.

Figure 2 shows a western blot.

Figure 3 shows a table.

Example 1: Combined detergent and protease treatment

5 A combined detergent and proteolytic enzyme treatment that reduces the titre of prion infectivity by up to a million fold is demonstrated.

This estimate is based upon a previously determined detection limit for the specific Western Blotting protocol used (Wadsworth *et al* Lancet 2001) which is a preferred assay method.

10 Using this assay method, it was determined that we can detect 5nl of a 10% w/v brain homogenate following PK digestion. In figure 1, each of the three lanes represents a sodium phosphotungstate precipitated pellet from 10ml of a 10% w/v brain homogenate. It can be seen that after treatment according to protocol A that PrP is only 15 just visible ie about 5nl equivalent. This initially contained 10,000,000 nl equivalents so the level of destruction is around 1 million fold.

20 The reagents used are water soluble, stable and of low toxicity. The protocol for their use is compatible with existing hardware for example as used in hospital decontamination departments for pre-washing and autoclaving instruments. Thus the invention provides for decontamination of prion infectivity from surgical instruments. Advantageously the methods of the present invention can be implemented using existing machinery.

25

Example 2: Destruction of PrPSc in aqueous samples.

This example describes methods by which the infectious material PrP^{Sc} can be deactivated in an aqueous suspension by serial exposure of the entity (in the example 30 the entity is infected brain tissue) to two proteolytic enzymes (ProteinaseK and pronase or pronase and papain) in the presence of a detergent.

The level of deactivation (in this example deactivation correlates with destruction) can be estimated from immunodetection of Western blots.

Preparation of tissue sample

5 Brain of vCJD-infected human frontal cortex was homogenised to 20% w/v in PBS Dulbecco's (GIBCO-BRL 14190-094) by passing the brain tissue through 18-gauge, 21-gauge and 23-gauge needles. The homogenate was diluted to 15% w/v with PBS, frozen in small aliquots and stored at -70°C.

10 **PROTOCOL A : Three-phase destruction of PrP^{Sc} from vCJD brain homogenate using ProteinaseK and pronase.**

15 (a) Treatment with detergent: sodium dodecyl sulphate (SDS). A 20 µl aliquot of 15% homogenate was measured into a screw-capped Eppendorf tube and 5 µl of 20% SDS was added to give a final concentration of 4% SDS. The mixture was heated for 15 minutes at 100°C and then cooled to room temperature. Cooling to 40 °C would be equally acceptable.

20 (b) Treatment with a first protease: ProteinaseK (PK). A solution of 40 µg/ml PK was prepared in double distilled water. A 5 µl aliquot of this enzyme solution was added to the 25 µl solution of detergent-treated homogenate produced by procedure (a), above. i.e. Final conc of ProteinaseK is 6.6ug/ml -The mixture was incubated at 40°C for 30 minutes.

25 (c) Treatment with a second protease: pronase. A solution of 2 mg/ml pronase was made up in 0.1 M TRIS/HCl pH 7.5. A 0.5 µl aliquot of this enzyme solution was added to the 30 µl solution of SDS- and PK-treated homogenate produced by serial procedures (a) and (b), above. i.e. Final conc of pronase is 33 ug/ml. The mixture was incubated at 40°C for 30 minutes.

30 **PROTOCOL B: Three-phase destruction of PrP^{Sc} from vCJD brain homogenate using pronase and papain.**

(a) Treatment with detergent: sodium dodecyl sulphate (SDS). A 20 μ l aliquot of 15% homogenate was measured into a screw-capped Eppendorf tube and 5 μ l of 20% SDS was added to give a final concentration of 4% SDS. The mixture was heated for 15 minutes at 100°C. And then cooled to between RT and 40°C before proceeding to next step.

(b) Treatment with a first protease: pronase. A solution of 0.5 mg/ml pronase was made up in 0.1 M TRIS/HCl pH 7.5. A 5 μ l aliquot of this enzyme solution was added to the 25 μ l solution of detergent-treated homogenate produced by procedure (a), above. I.e. Final conc of pronase is 83ug/ml. The mixture was incubated at 40°C for 30 minutes.

(c) Treatment with a second protease: papain. A solution of 0.4 mg/ml papain was made up in 0.1 M TRIS/HCl pH 6.0. A 0.5 μ l aliquot of this enzyme solution was added to the 30 μ l solution of SDS- and pronase-treated homogenate produced by serial procedures (a) and (b), above. Final conc of papain is 6.5 ug/ml. The mixture was incubated at 40°C for 30 minutes.

Detection of PrP^{Sc} by Western Blot

The materials from the SDS/PK/pronase (Protocol A) and the SDS/pronase/papain (Protocol B) treatments described above were submitted to Western blot analysis, see Figure 2. The blots were visualized using the antibodies ICSM 18 and ICSM 35 to detect any remaining PrP^{Sc} in the samples. Using either antibody, there was no detectable PrP^{Sc}.

Following various enzymatic treatments detailed above the samples were subjected to SDS-PAGE and visualised by Western blotting and detection with the antibody ICSM35. The first lane (Lane 1 - untreated material) is a control sample of untreated material.

The second lane is treated with just 4% w/v SDS at 100°C for 15 minutes (Lane 2 - 4% SDS 100°C 15mins).

The third lane shows SDS and papain treatment, (Lane 3 - 4% SDS & papain : two steps corresponding to step 1 & step 3 in protocol B)

5 The fourth lane combines SDS pronase then papain (Lane 4 -Protocol B ie three steps.)

The fifth lane is SDS followed by PK alone (Lane 5 -the times, concentrations and temperatures are as steps 1 & 2 of Protocol A)

10

The sixth lane shows SDS and sequential PK then pronase - (Lane 6 - Protocol A)

The seventh lane shows SDS and sequential pronase then PK - (Lane 7 Protocol A but with steps 2 & 3 reversed)

15

The final eighth lane is SDS and pronase alone (Lane 8 - just steps 1 & 2 from protocol B).

20

Similar results are observed when ICSM18 is used for visualisation of the Western blot.

25

A note on interpretation of figure 2: All the bands are PrP. The control lane contains total PrP, ie both PrP^C and PrP^{Sc}. It is not possible to define which are PrP^{Sc} *a posteriori* as these are functionally defined. However, in the absence of any PrP we can then say that PrP^{Sc} is absent. It is clear from studying figure 2 that the methods of the present invention lead to significant prion decontamination. For example, with Protocol B (Lane 4) there are some immunoreactive bands remaining. These are PrP, (most likely to be PrP^{Sc}) however, it is plain that the level has been drastically reduced from the starting material, thereby demonstrating significant prion decontamination according to the present invention.

30

Example 3: Destruction of prion infectivity on surgical steel

In this example, the application of the methods of the present invention to the destruction of prion infectivity on surfaces (in this example the surfaces are surgical steel), so demonstrating the applicability to the decontamination of surgical instruments. Again, advantageously the methods of the invention may be implemented in pre-washing procedures carried out in hospital decontamination departments. Small samples of this surgical steel are implanted into transgenic mice to bioassay for residual infectivity.

10

This experiment was designed to demonstrate the efficacy of disinfection provided by enzymatic treatment compared to existing treatment methods. 5mm x 0.15mm steel wires were incubated for 30 minutes with a 20% homogenate prepared from the brain of a CD1 mouse terminally sick with Rocky Mountain Laboratories (RML) scrapie. 15 These wires were then dried without washing and inserted into the brains of Tg20/ZH1 transgenic mice (which had been bred to overexpress the normal prion protein PrP^c).

Before insertion wires were either not subjected to disinfection (positive control group), incubated for 90 minutes at RT with existing disinfection compounds (LpH, LpHse, Endozyme Plus), autoclaved or treated with enzymes (with or without 20 autoclaving).

The enzymatic treatment comprised heating at 100°C in 4% SDS for 15 minutes, followed by cooling to 40°C followed by the addition of pronase followed by papain. Each enzymatic incubation was performed at 40°C for 30 minutes. (i.e. protocol B) (Protocol A (SDS, PK, Pronase) is also suitable for decontaminating surgical 25 instruments.)

The wires were then briefly rinsed with cool SDS. A negative control group was provided by inserting wires that had not been incubated with RML homogenate.

To measure the infectivity of the RML homogenate in which the wires were incubated, 30µl of different dilutions were inoculated intra-cerebrally into further groups of mice.

This confirmed that all of the homogenate used was infectious. It also quantitatively determines the infectious titre of the homogenate.

In order to test the effect of exposing steel to intact brain (as opposed to a 20% homogenate) one group of mice had wires inserted that had been dipped for 30 minutes in the brain of a terminally sick CD1 mouse. These wires were briefly rinsed in Phosphate Buffered Saline - Dulbecco's (PBS) and then dried before insertion.

Detailed Experimental procedures:

Materials

10

Pronase (SIGMA CODE P5147; 1.16 mg/ml in 0.1 M TRIS/HCl pH 7.5)
Papain (SIGMA CODE P3375; 0.21 mg/ml in 0.1 M TRIS/HCl pH 6.0)

Implantation of uninfected wire

15

Single segments of untreated wires were manually implanted into the brain of Tg20 mice by using a G26 needle as guide.

(Group A)

Dipping and implantation of dipped wires

20

Ten wire segments (5 mm) were transiently inserted into the brain of a RML-infected CD1 mouse (right side of bregma) for 30 min. "Dipped" wire segments were briefly rinsed in 50 ml PBS Dulbecco's (GIBCO-BRL 14190-094) using a 50 ml FALCON tube and dried for 30 min. on a petri dish at room temperature. A single segment was manually implanted into the brain of each mouse by using a G26 needle as guide.

25

(Group B)

Preparation of 20 % (w/v) RML brain homogenate:

Brain homogenate (20 % w/v) was prepared in PBS Dulbecco's (GIBCO-BRL 14190-094) by passing the brain through 18-g, 21-g and 23 gauge needles. Brain of RML-infected CD1 mouse used for dipping (wet weight: 400 mg) was homogenized in 1 ml

30

PBS. Sample was adjusted to a final volume of 2 ml with PBS. The total homogenate

called 20 % (w/v) (without any centrifugation step) and used immediately for the next step. Aliquots were frozen at -20°C.

Exposure of wire to 20 % (w/v) RML brain homogenate:

5 Groups of 20 wire segments were exposed to freshly prepared 20% w/v RML brain homogenate (0.1 ml) in a 1.5 ml Safe-lock Eppendorf tube and incubated for 30 min at 22°C with agitation. After incubation, brain homogenate was drawn off by a pipette and the exposed wires transferred directly to a petri dish. Wires were dried for 30 min at room temperature. Single segments were manually implanted into the brain of each

10 mouse by using a G26 needle as guide.

(Group C)

Treatments for decontamination:

Treatments with LpH, LpHse and Metal Pro

15 LPH/LPHse (Steris, Steris House, Jays Close, Viables, Basingstoke, Hants RG22 4AX UK), Endozyme Plus (The Ruhof Corporation : 393 Sagamore Avenue : Mineola, NY 11501). LPH and LPHse are proprietary compounds designed for disinfection of worktops and similar surfaces that may be exposed to contamination. Endozyme Plus is marketed for disinfection of medical instruments.

20 Three groups of twenty wire segments exposed to brain homogenate (as described above) and transferred into a Eppendorf tube containing 0.2 ml of solution (LpH, LpHse, or Metal Pro; each at 10% v,v in double distilled water. They were incubated for 90 min at room temperature. (Solutions were prepared prior to use by addition of 25 0.15 ml stock to 1.35 ml ddwater). Wires were briefly rinsed with 50 ml and 25 ml PBS.

LpH (Group D)

LpHse (Group E)

Metal Pro (Group F)

30

Autoclaving

Twenty wire segments exposed to brain homogenate (see 3) were put in a sealed autoclave bag and autoclaved on an uncovered autoclave tray at 121°C for 20 min and at 134°C for 30 min, respectively. Autoclaved wire segments were implanted into the brains of Tg20 mice (single segment each).

5 **121°C (Group G)**

134°C (Group H)

Enzymatic treatment alone or followed by autoclaving

Twenty wire segments exposed to brain homogenate (see 3) were transferred into an 10 Eppendorf tube containing 4% w/v SDS in double distilled water and boiled at 100°C for 15 min. Eppendorf tubes were cooled down to 40°C in the presence of 4% SDS and liquid was removed by pipetting. 100µl of pronase solution (1.16mg/ml) was added to the same Eppendorf tube and incubated at 40°C for 30 min. Solution was removed and 100µl of papain solution (0.21mg/ml) was added to the same Eppendorf tube and 15 further incubation at 40°C for 30 min. Wires were briefly rinsed with 50 ml and 25 ml PBS. One group of treated wires was assayed for infectivity by permanent implantation of a single segment into the brain of Tg20 mouse (Group I)

20 Two groups of such treated wires were subjected to autoclaving in parallel to the sample as described in 4.1. and later a single segment implanted into the brain of each Tg20 transgenic mouse.

Enzymatic treatment and 121°C 20 min (Group J)

Enzymatic treatment and 134°C 20 min (Group K)

25

Results are shown in table 1 (see figure 3)

It is thereby demonstrated that the methods of the present invention result in significant prion decontamination.

30

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and

compositions of the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the present invention. Although the present invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in biochemistry and biotechnology or related fields are intended to be within the scope of the following claims.

CLAIMS

1. A method for prion decontamination of an entity comprising
 - (i) contacting said entity with a detergent,
 - (ii) contacting said entity with a protease, and optionally
 - 5 (iii) autoclaving said entity.
2. A method according to claim 1 wherein the protease is selected from the group consisting of ProteinaseK, papain, pronase, and bromelain.
- 10 3. A method according to claim 2 wherein the protease is pronase.
4. A method according to claim 2 wherein the protease is ProteinaseK.
5. A method for prion decontamination comprising
 - 15 (i) contacting an entity to be decontaminated with a detergent,
 - (ii) contacting said entity with a first protease,
 - (iii) contacting said entity with a second protease, and optionally
 - (iv) autoclaving said entity.
- 20 6. A method according to claim 5 wherein the first and second proteases are selected from the group consisting of ProteinaseK, papain, pronase, and bromelain.
7. A method according to claim 6 wherein the first protease is pronase and the second protease is papain.
- 25 8. A method according to claim 6 wherein the first protease is ProteinaseK and the second protease is pronase.
9. A method according to claim 6 wherein the first protease is pronase and the 30 second protease is ProteinaseK.

10. A method according to any preceding claim wherein the detergent is SDS.
11. A method according to any preceding claim wherein said entity comprises a surface.
- 5 12. A method according to claim 11 wherein said surface is a surface of a medical instrument.
- 10 13. A method according to claim 11 or claim 12 wherein said surface comprises metal.
14. A method according to claim 13 wherein said metal is steel.
15. A kit comprising detergent and a protease selected from the group consisting of ProteinaseK, papain, pronase, and bromelain.
16. A kit according to claim 15 comprising two or more said proteases.
17. A kit according to claim 15 or 16 wherein said detergent is SDS
- 20 18. A composition comprising an ionic detergent and one or more proteases selected from the group consisting of ProteinaseK, papain, pronase, and bromelain.
19. A composition according to claim 18 comprising two or more said proteases.
- 25 20. A composition according to claim 18 or 19 wherein said detergent is SDS.

ABSTRACTPRION DECONTAMINATION

The present invention relates to methods for prion decontamination comprising (i) contacting an entity to be decontaminated with a detergent, (ii) contacting said entity with one or more protease(s), and optionally (iii) autoclaving said entity. The invention further relates to kits comprising detergents and proteases for prion decontamination, and compositions comprising detergents and proteases for prion decontamination.

Figure 1

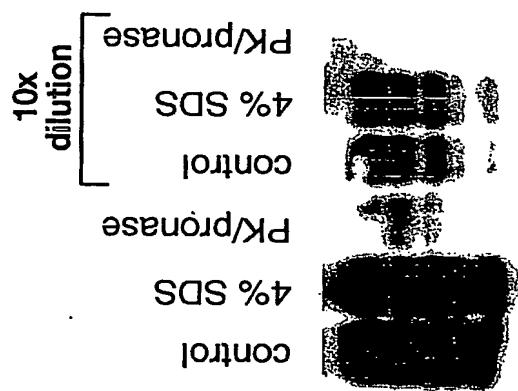


Figure 2 Western Blot detection of remaining PrP^{Sc}

MW (kDa) C SDS Pa Pr/Pa PK PK/Pr Pr/PK Pr

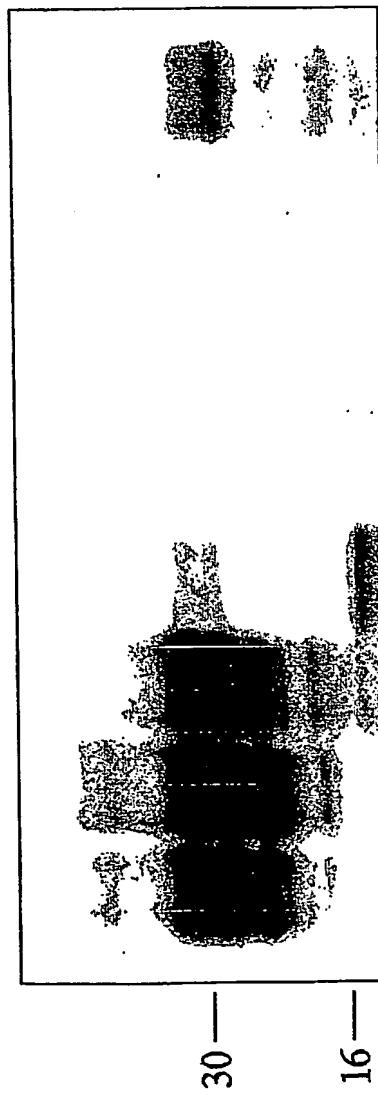


Figure 3Table 1

Inoculum	Sick/Total	Time to Death \pm s.d.
5mm Steel Wires		
Uninfected		
Untreated (Group A)	0/6	
Infected (dipped for 30min in infected brain)		
Briefly rinsed, then dried (Group B)	5/5	86 \pm 9d
Infected (incubated with 20% homogenate)		
Unwashed, dried (Group C)	4/4	72 \pm 4d
LpH (10%, 90mins, RT) (Group D)	5/5	91 \pm 5d
LpHse (10%, 90mins, RT) (Group E)	3/5	73 \pm 2d
Endozyme Plus (10%, 90mins, RT) (Group F)	5/5	84 \pm 5d
Autoclaving (121°C, 20 mins) (Group G)	0/6	
Autoclaving (134°C, 30 mins) (Group H)	0/4	

Pronase/Papain/SDS	(Group J)	0/5	
Pronase/Papain/SDS + Autoclaving (121°C, 20 mins)	(Group J)	0/5	
Pronase/Papain/SDS + Autoclaving (134°C, 30 mins)	(Group K)	0/5	

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

BLACK BORDERS

IMAGE CUT OFF AT TOP, BOTTOM OR SIDES

FADED TEXT OR DRAWING

BLURRED OR ILLEGIBLE TEXT OR DRAWING

SKEWED/SLANTED IMAGES

COLOR OR BLACK AND WHITE PHOTOGRAPHS

GRAY SCALE DOCUMENTS

LINES OR MARKS ON ORIGINAL DOCUMENT

REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY

OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.